

Supporting Information

High Expression of Dehydroascorbate Reductase Improves Salt Tolerance, Environmental Adaptability, and Productivity in *Oryza sativa*

Young-Saeng Kim ^{1,*}, Seong-Im Park ^{2,†}, Jin-Ju Kim ², Sun-Young Shin ³, Sang-Soo Kwak ⁵, Choon-Hwan Lee ⁶, Hyang-Mi Park ⁷, Yul-Ho Kim ⁸, Il-Sup Kim ^{4,*} and Ho-Sung Yoon ^{2,3,4,*}

¹ Research Institute of Ulleung-do & Dok-do, Kyungpook National University, Daegu 41566, Korea; kyslhh1228@hanmail.net

² School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu 41566, Korea; sheep91528@naver.com (S.-I.P.); deenamon@naver.com (J.-J.K.)

³ Department of Biology, College of Natural Sciences, Kyungpook National University, Daegu 41566, Korea; snowmooa@hanmail.net

⁴ Plant Systems Engineering Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Korea; sskwak@kribb.re.kr

⁵ Department of Molecular Biology, Pusan National University, Busan 46241, Korea; chlee@pusan.ac.kr

⁶ National Institute of Crop Science, Rural Development Administration, Jeonju 54875, Korea; parkhm2002@korea.kr

⁷ Highland Agriculture Research Institute, National Institute of Crop Science, Rural Development Administration, Pyeongchang 25342, Korea; kimyuh77@korea.kr

⁸ Advanced Bio-Resource Research Center, Kyungpook National University, Daegu 41566, Korea

* Correspondence: 92kis@hanmail.net (I.-S.K.); hsy@knu.ac.kr (H.-S.Y.)

† These authors contributed equally to this work.

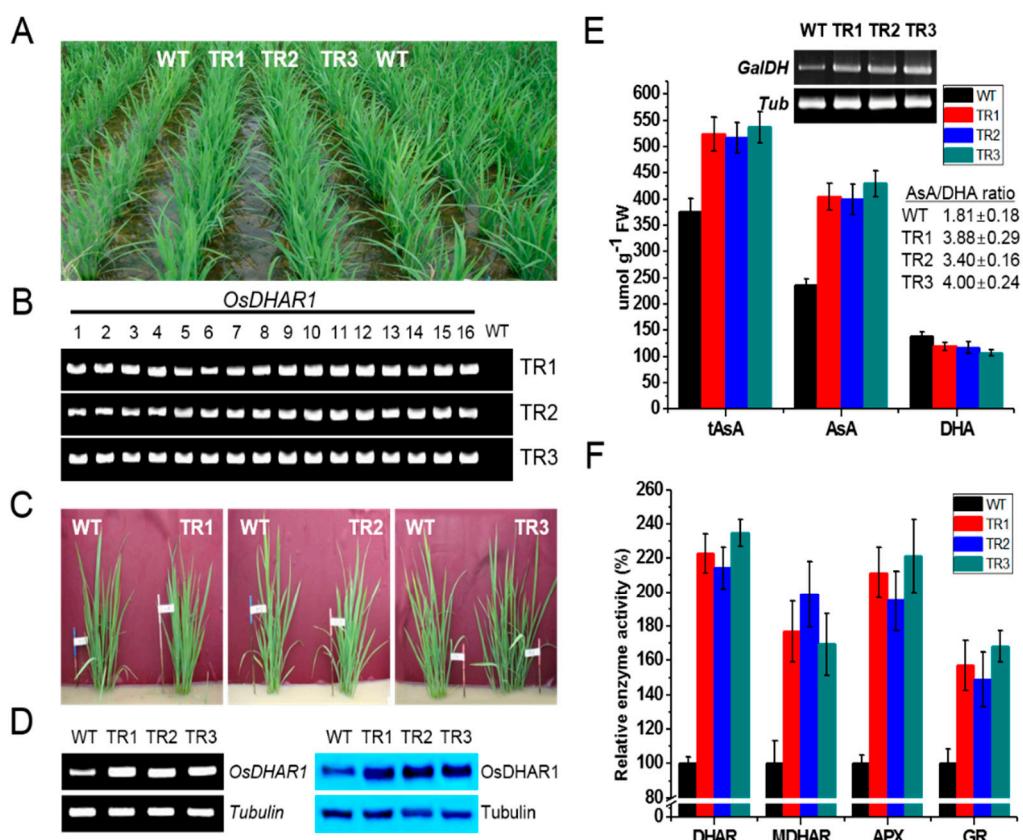


Figure S1. Environmental adaptability of *OsDHAR1*-expressing transgenic plants in a natural paddy field. (a) Phenotype analysis of TR and WT plants in the vegetative stage. (b) PCR confirmation of *OsDHAR1* expression in three separate TR plants (TR1-TR3). (c) Phenotype of TR and WT plants grown for 4 weeks in the paddy fields. (d) Confirmation of *OsDHAR1* expression using semi-quantitative RT-PCR and western blot analysis. Signal intensity in WT plants was due to endogenous *DHAR1* expression. *Tubulin* (*Tub*) and corresponding protein were used as loading controls. WT, wild-type rice; TR, transgenic rice. (e) Ascorbate pool and *GalDH* expression in TR and WT plants 4 weeks after transplantation. (f) Enzymatic activity of TR and WT plants grown for 4 weeks in the paddy field.

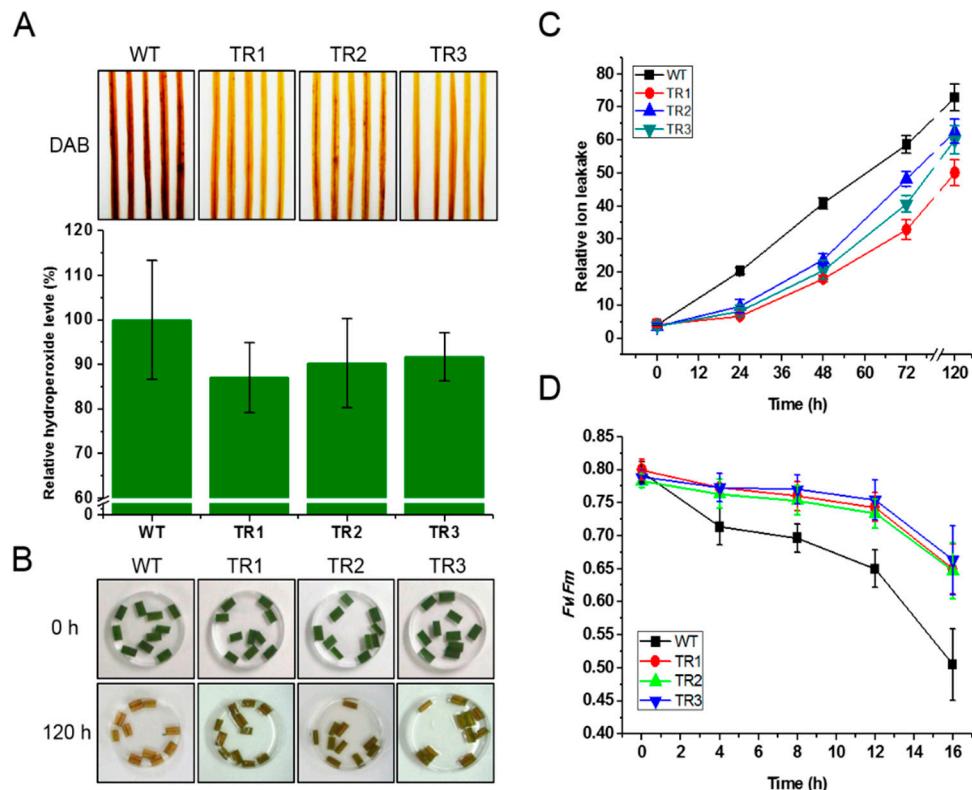


Figure S2. Redox state and photosynthetic capacity of *OsDHAR1*-expressing transgenic plants. (a) 3,3-Diaminobenzidine (DAB) staining phenotype and relative hydroperoxide level in leaves from TR and WT plants treated with 50 μ M methyl viologen (MV) for 12 h. (b) Phenotype analysis of cells damaged in leaf discs of TR and WT plants exposed with 10 μ M MV for 120 h. (c) Relative ion leakage in TR and WT plants treated with 10 μ M MV from 0 h to 120 h. (d) Changes in chlorophyll fluorescence (F_v/F_m) in three independent TR and WT plants in response treatment with 10 μ M MV.

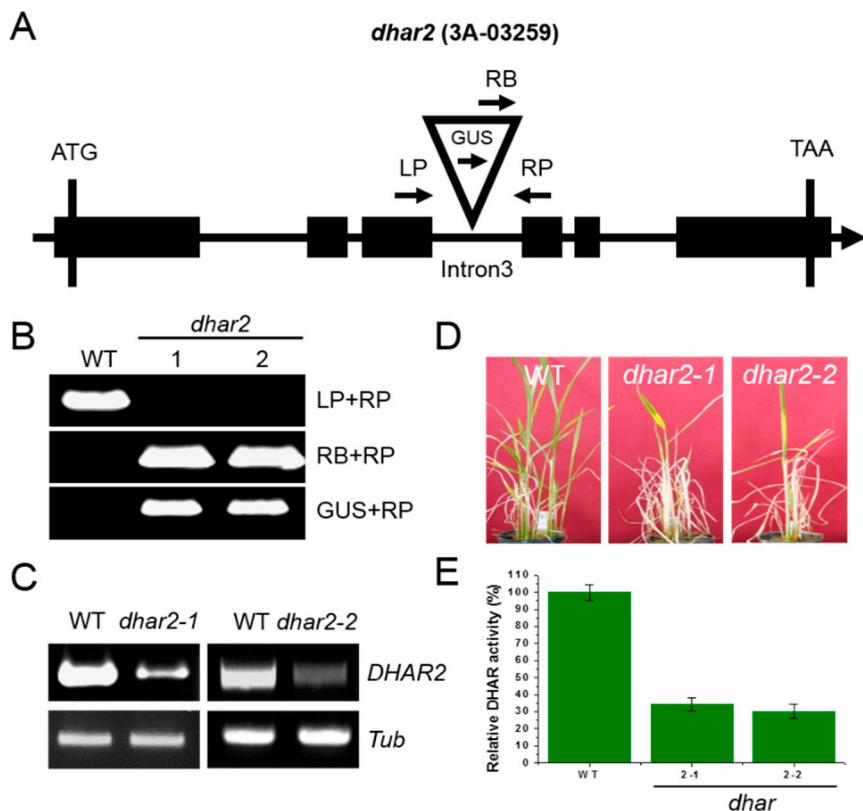


Figure S3. Characterization of *OsDHAR2* knockdown mutants. **(a)** Insertion positions of T-DNA spanning the mutation site in *dhar2* (3A-03259). **(b)** PCR-based confirmation of *dhar2* mutant lines. **(c)** Evaluation of *DHAR2* expression in *dhar2* plants using semi-quantitative RT-PCR. **(d)** Relative DHAR activity in *dhar2* and WT plants. **(e)** Phenotype of *dhar2* and WT plants treated with 100 mM NaCl for 25 days.

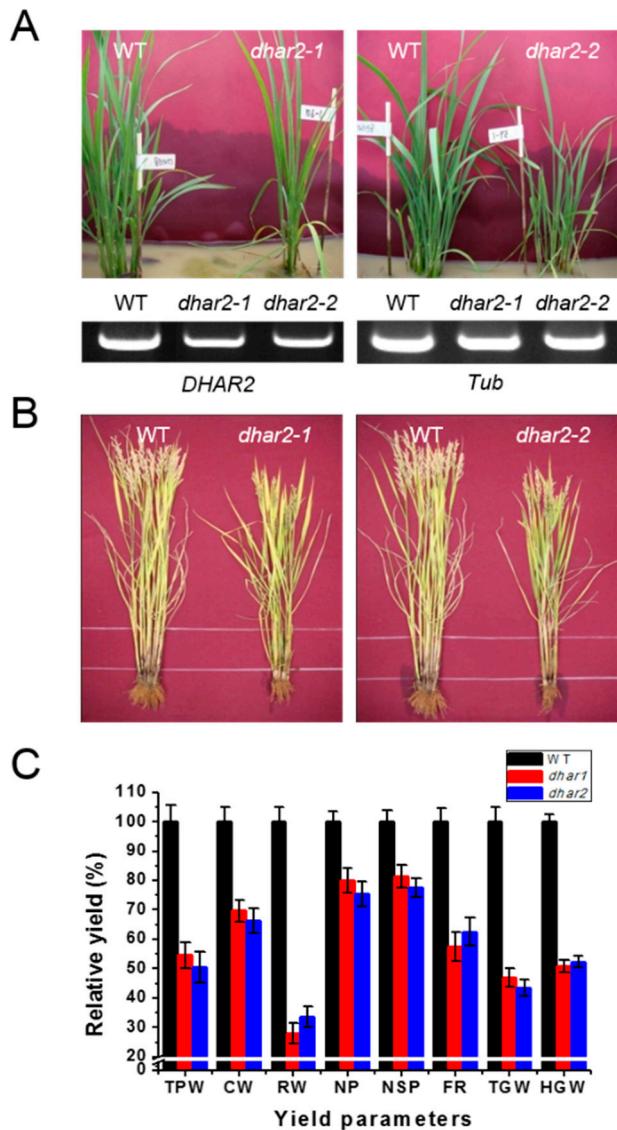


Figure S4. The poor agronomic parameters of the *dhar2* plants grown in the paddy field. **(a)** Phenotype and *DHAR2* expression in *dhar2* and WT plants grown for 4 weeks in the paddy fields. **(b)** Phenotypes of *dhar2* and WT plants at the late reproductive stage. **(c)** Agronomic traits of *dhar2* and WT plants.

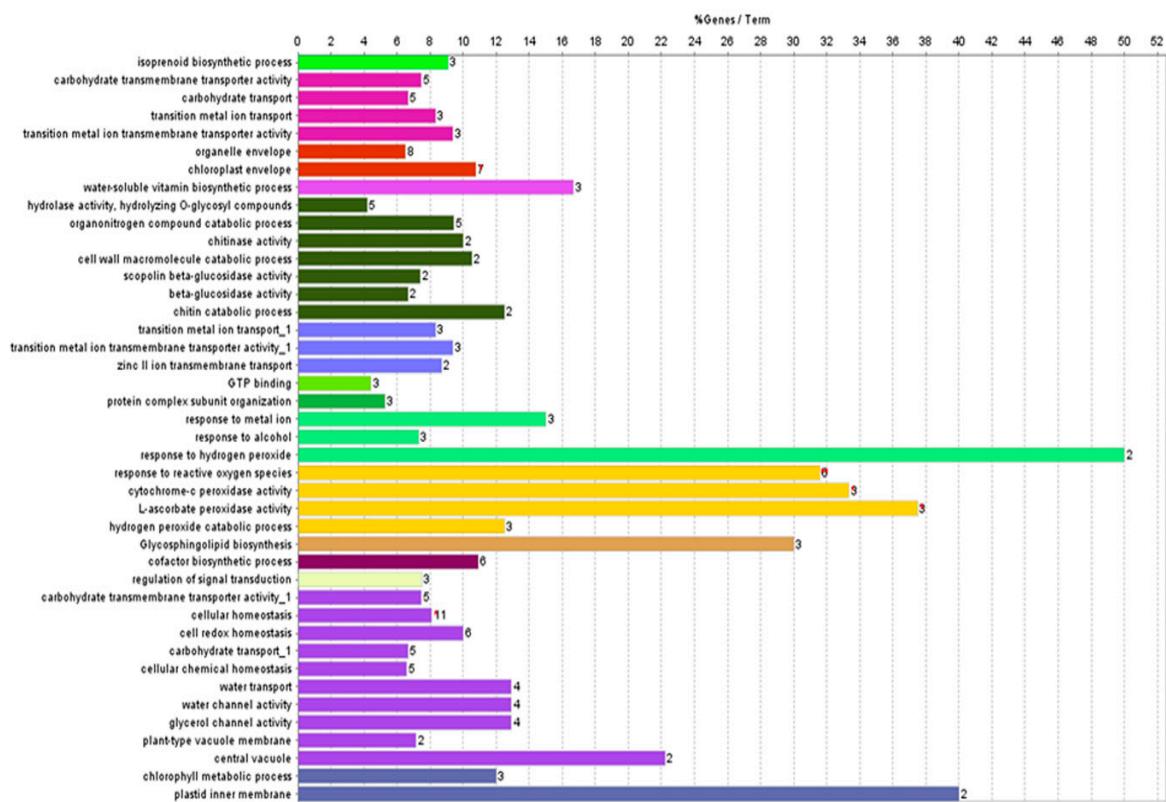


Figure S5. Gene ontology of the genes upregulated in transgenic rice (TR1) plants.

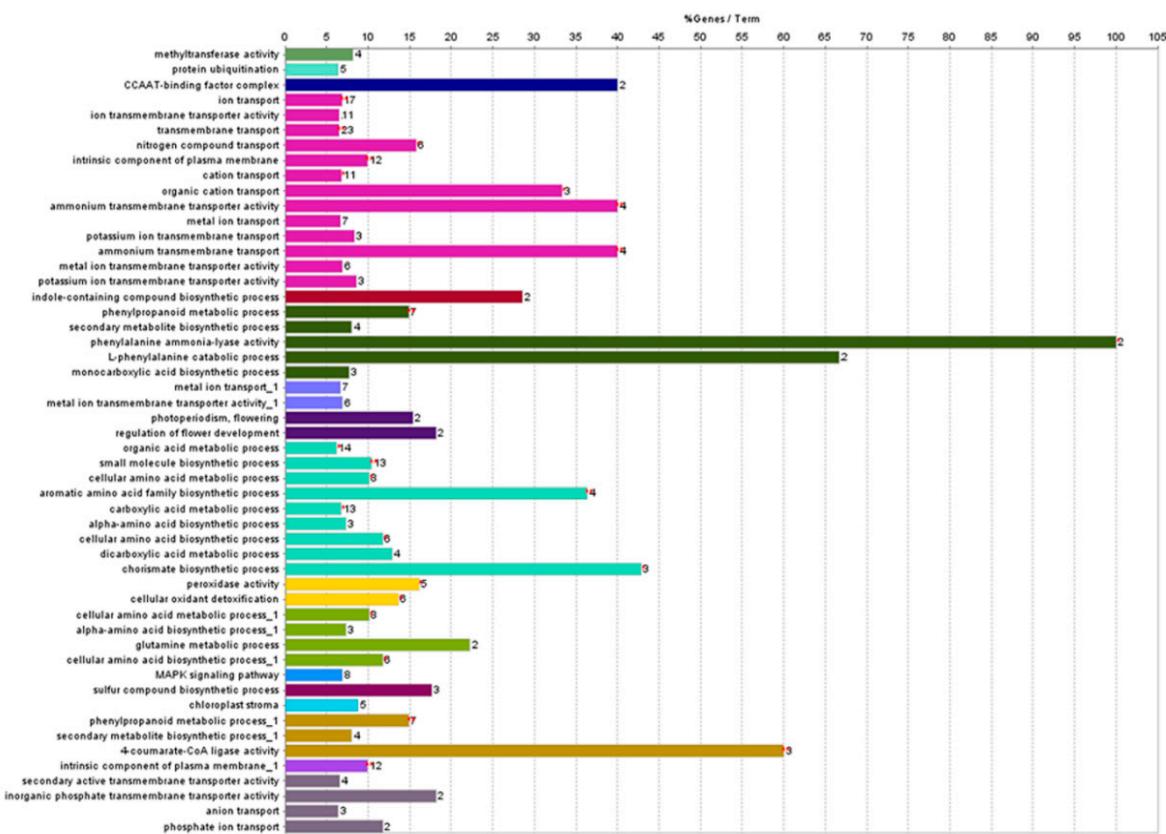


Figure S6. Gene ontology of the genes downregulated in transgenic rice (TR1) plants.

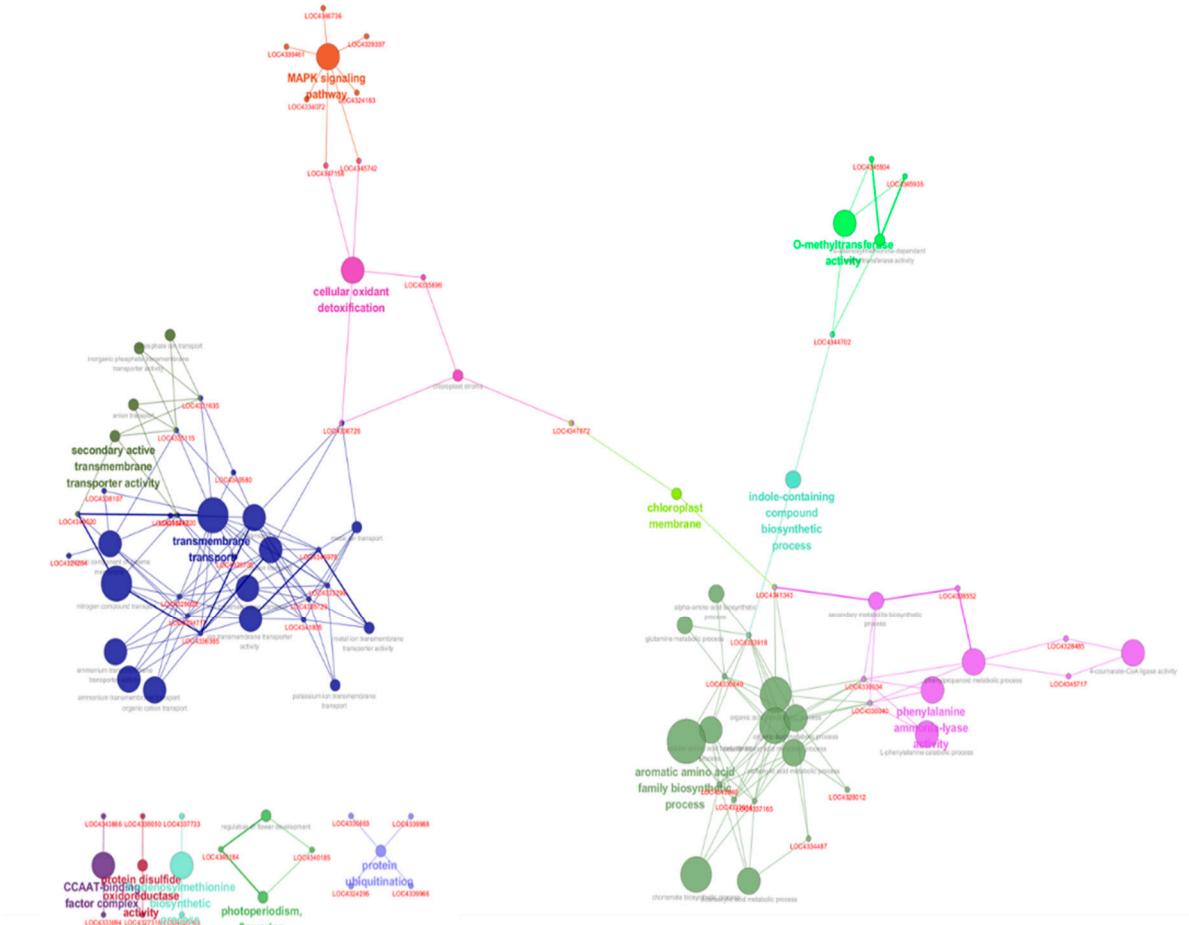


Figure S7. Genetic network of the genes downregulated in transgenic rice (TR1) plants.

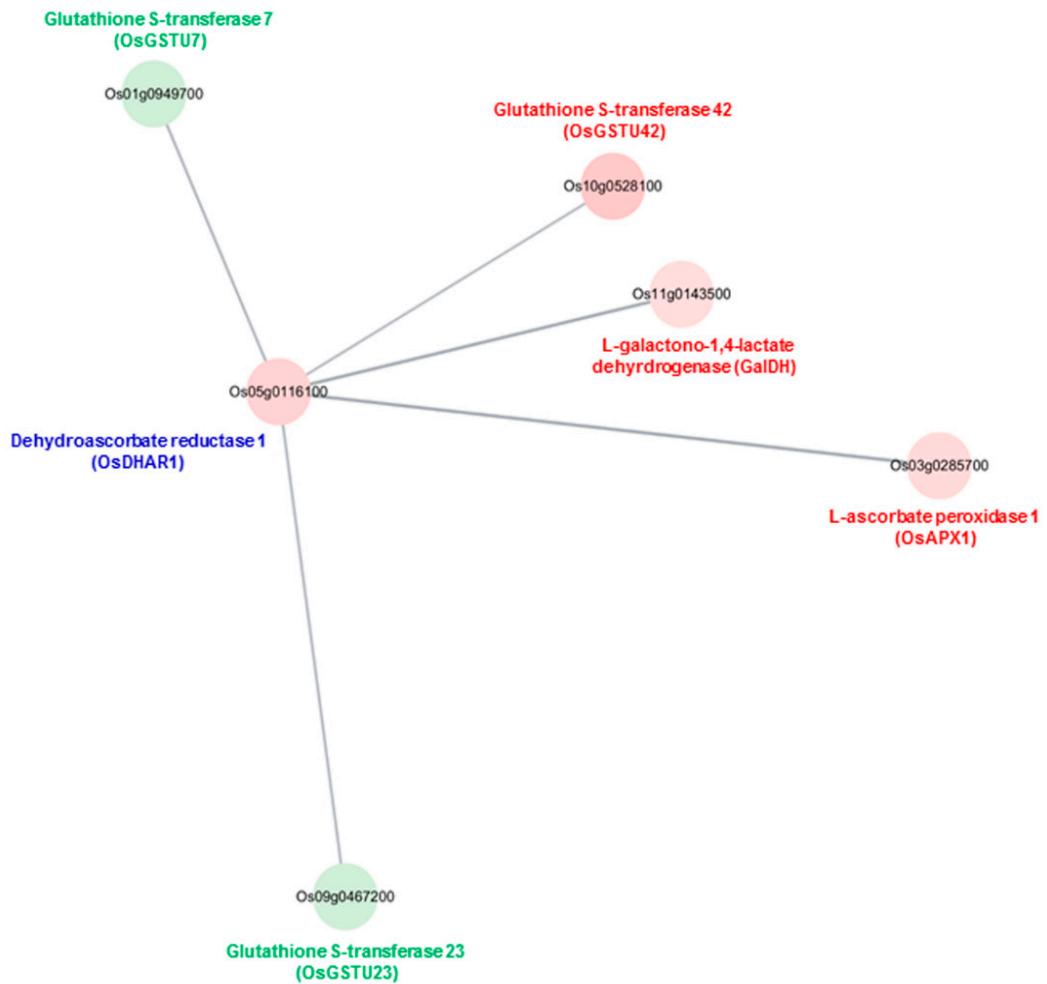


Figure S8. Genetic interaction map of *OsDHAR1* and other genes in transgenic rice (TR1) plants. The up- and downregulated genes are marked in red and green, respectively.

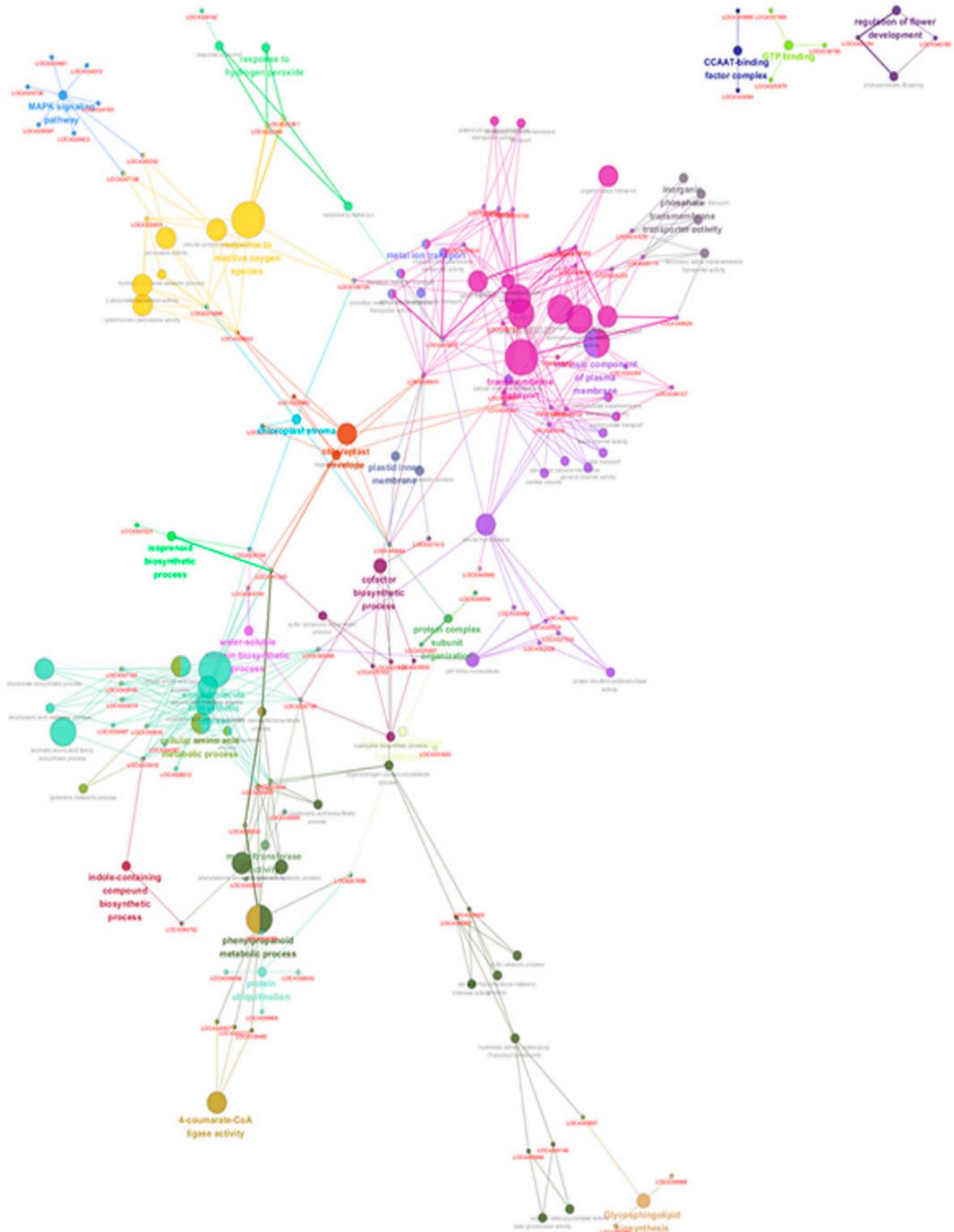


Figure S9. Genetic network of up- and downregulated genes in transgenic rice (TR1) plants.

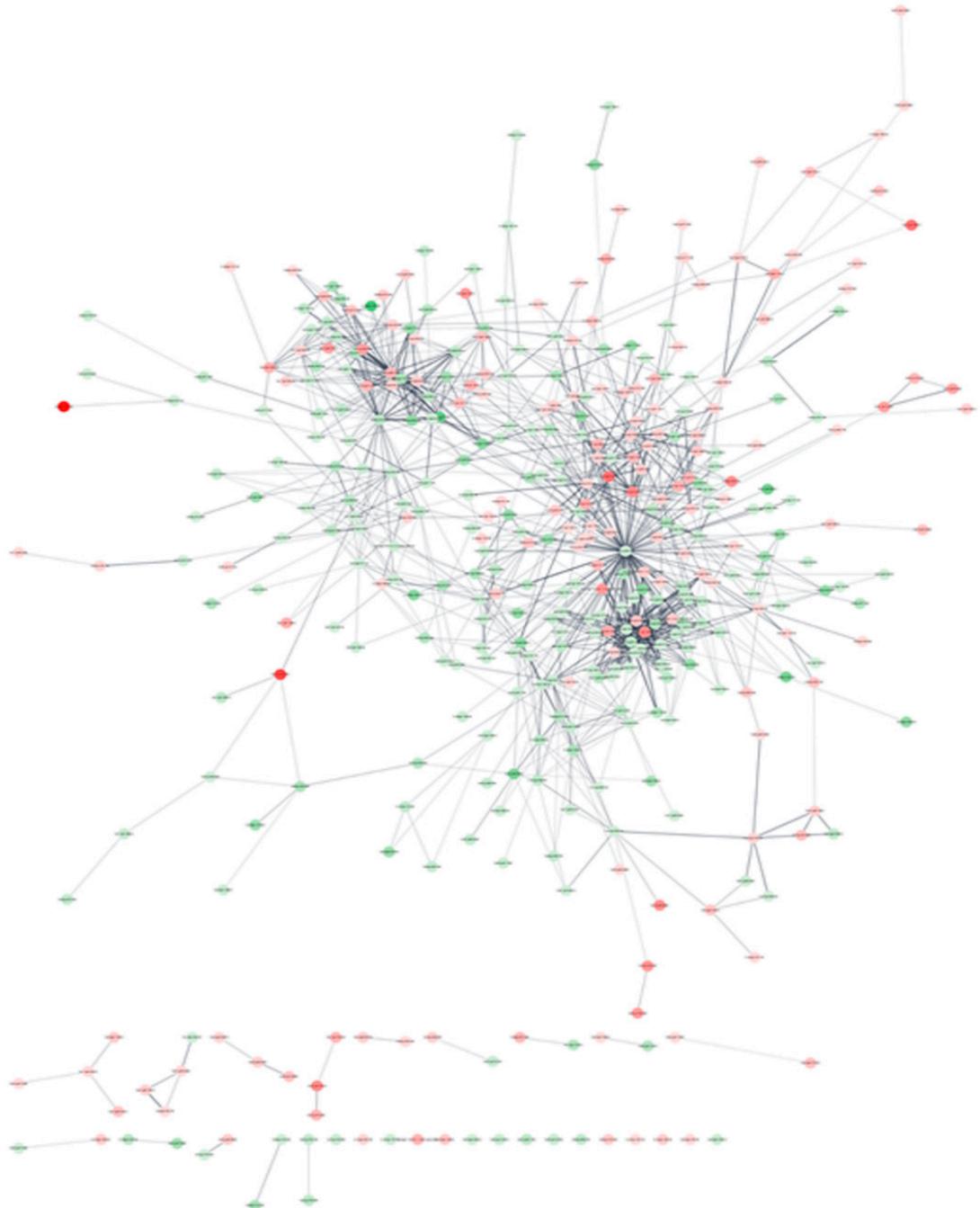


Figure S10. Gene to gene network of differentially expressed genes.

Table S1. Oligonucleotides used in the present study.

Oligo name	Oligonucleotide sequences (5'-3')
OsDHAR-FC	TCGCCGCC <u>ATGGCGTGGAGGTGTGCG</u>
OsDHAR-RC	CCAAGCAAG <u>GGTACCTGCAGGCTGCATCTCCATTATTC</u>
SWPA2-F1	TGCCTTCATACGCTATTATTGCTTGG
OsDHAR-R1	CCTTGCTCTTCAAGAACGTTGTGAAGC
OsDHAR-F2	ATGGCGTGGAGGTGTGCGTCAAGG
OsDHAR-R2	CCTTGCTCTTCAAGAACGTTGTGAAGC
LP	AAGCGCAATTTACAGCTGAG
RP	CCGTTAATAAATGGACCCTGC
RB	TTTCGCGATCCAGACTGAATG
GUS	ATGTGGAGTGAAGAGTATCAGTGTGC
ADA1	GCGTAATACGACTCACTATAGCAATTAAACC
ADA2	GA ^T CTCACTATAGCAATTAAAC
LB1	GTGTGAGTAGTTCCCAGATAAGGAAATTAGG
LB2	CGCTCATGTGTTGAGCATA
RB1	CCCGCACCGATCGCCCTTCCCA
RB2	GCAGCTTGAGCTTGGATCAGA
GalDH-F	TACAGGGAAGGGTTCGACTTCAGC
GalDH-R	AGCACATAAGTAGATGCTCAATG
Tub-F	TACCGTGCCCTTACTGTTCC
Tub-R	CGGTGGAATGTCACAGACAC

Underlined nucleotides indicate the sites recognized by the restriction enzymes. Primer sequences were designed based on the information hosted on RAP-DB (<http://rapdb.dna.affrc.go.jp/>).

Table S2. Copy number and location of the *OsDHAR1* transgene.

TR	Chromo	Matching	Chr_start	Chr_end	Type	Gene ID	Description
No.	some	(%)					
TR1	Chr03	100	14536694	14536533	Intergenic	Os03g0360000	Similar to upstream 3.008 arginine
					kb		decarboxylase
TR2	Chr08	100	27527684	27527785	Intron	Os08g0547500	Similar to kinesin-like protein NACK1

TR3	Chr04	100	27722481	27722149	Exon	Os04g0543700	Similar to serine protease (fragment)
-----	-------	-----	----------	----------	------	--------------	---------------------------------------

Table S3. Genes upregulated in paddy field-grown transgenic rice (TR1) plants.

Gene symbol	Intensity	Gene information
Os03g0115800	223.33	Conserved hypothetical protein
Os11g0206000	210.14	Conserved hypothetical protein
Os03g0115800	149.52	Conserved hypothetical protein
Os07g0153100	114.97	Hypothetical protein
Os01g0382000	38.57	Similar to pathogenesis-related protein PRB1-2 precursor
Os06g0229000	13.64	Similar to FtsH protease (VAR2) (Zinc dependent protease)
Os12g0628600	9.19	Similar to thaumatin-like pathogenesis-related protein 3 precursor
Os11g0113700	7.41	Serine/threonine protein kinase, abiotic stresses
Os06g0685300	6.84	Similar to isoflavone reductase homolog IRL
Os11g0530600	6.46	Similar to chalcone synthase C2 (EC 2.3.1.74)
Os12g0569500	5.98	Thauatin, pathogenesis-related family protein
Os04g0249600	5.85	Similar to senescence-associated protein DIN1
Os01g0314800	5.02	Late embryogenesis abundant protein 3 family protein
Os05g0160300	4.95	Bifunctional inhibitor/plant lipid transfer protein/seed storage domain containing protein
Os01g0136100	4.48	16.9 kDa class I heat shock protein 1
Os04g0612400	4.33	Similar to root-specific protein; RCc2 protein
Os04g0612500	4.32	Similar to 36.4 kDa proline-rich protein
Os04g0612400	4.20	Protease inhibitor/seed storage/LTP family protein precursor
Os01g0184100	4.07	Similar to 17.5 kDa class II heat shock protein
Os01g0368900	4.04	Similar to glutaredoxin-C1; glutaredoxin 4
Os01g0124000	3.92	Similar to bowman birk trypsin inhibitor
Os03g0431200	3.86	Similar to N-acetyl-glutamate synthase
Os10g0491000	3.83	Plant basic secretory protein family protein
Os10g0490800	3.67	Similar to secretory protein
Os03g0663400	3.55	Similar to thaumatin-like protein; OsLP
Os12g0541700	3.52	Similar to rapid alkalinization factor 2
CB647177	3.13	Photosystem II 10 kDa polypeptide, chloroplast precursor
Os10g0528100	3.06	Similar to glutathione S-transferase GST 42; OsGSTU24
Os05g0413200	2.89	Beta-tubulin; OsTUB6
Os03g0820500	2.84	Actin depolymerizing factor 3; OsADF3
Os02g0758000	2.77	Similar to low molecular weight heat shock protein precursor (mitochondrial small heat shock protein 22); OsHsp24.1
Os01g0279100	2.71	Similar to basic leucine zipper transcription factor CAT103
Os06g0625400	2.68	Similar to metalloendopeptidase

Os01g0186000	2.67	Similar to WRKY transcription factor 10; OsWRKY10
Os02g0258900	2.65	Similar to molybdopterin biosynthesis CNX2 protein
Os04g0688100	2.65	Peroxidase; Prx59
Os06g0650900	2.62	Heat shock protein DnaJ family protein; OsDjC56
Os05g0582000	2.58	Plant basic secretory protein domain containing protein
Os10g0548600	2.56	Beta-expansin precursor; OsEXPB9
Os03g0218500	2.52	Similar to 70kD heat shock protein
BQ907184	2.49	Photosystem II 10 kDa polypeptide, chloroplast precursor
Os03g0267000	2.45	Low molecular mass heat shock protein Oshsp18.0; OsHSP18.0-Cl
Os01g0232000	2.44	Major intrinsic protein family protein; probable aquaporin TIP4.3 (OsTIP4;3)
Os05g0116100	2.44	Dehydroascorbate reductase 1; OsDHAR1
Os03g0843800	2.43	Methyl chloride transferase; harmless to ozone layer 1 (OsHOL1)
Os04g0691900	2.42	Chaperonin Cpn60/TCP-1 family protein
Os03g0180900	2.42	Jasmonate ZIM-domain protein 2; OsJAZ2
Os03g0848700	2.38	Brown planthopper resistance-14; Bph14
Os01g0111100	2.38	Cyclophilin-like domain containing protein; OsCYP26-2
Os01g0838350	2.37	Phosphate-limitation inducible gene 1; OsPII
Os02g0629200	2.36	Probable aquaporin PIP2-2; OsPIP2;2
Os01g0583300	2.31	Zinc finger, C2H2, LYAR-type domain containing protein; Os_F0727
Os03g0161900	2.31	Heat stress transcription factor A2d; HSfA2d
Os04g0345400	2.30	Terpene synthase 24; OsTPS24
Os03g0782500	2.30	Phytochrome-interacting factor-like protein 1; OsPIL1
Os10g0506100	2.25	Heavy metal transport/detoxification protein domain containing protein
AK100788.1	2.24	ABA/WDS induced protein
Os01g0939500	2.23	Similar to eukaryotic peptide chain release factor subunit 1-2 (eRF1-2)
Os02g0553200	2.23	Ascorbate peroxidase 8; OsAPx8
Os04g0423400	2.20	Abiotic stress responsive 2 (OsASR2); ABA-, stress and ripening-induced protein 4 (OsASR4)
Os01g0117900	2.20	Similar to nodulin-like protein
Os11g0222225	2.18	Serpin domain containing protein
Os02g0707000	2.17	Monodehydroascorbate reductase 1; OsMDHAR1
Os01g0284700	2.17	Similar to peptidyl-prolyl cis-trans isomerase; OsCYP20-3
Os11g0592000	2.15	Pathogenesis-related protein 4c; OsPR4c
Os08g0479300	2.13	Cyclin, A/B/D/E domain containing protein
Os03g0285700	2.11	L-ascorbate peroxidase 1; OsAPX1
Os03g0745000	2.10	Heat stress transcription factor A2a; HSfA2
Os01g0232000	2.09	Probable aquaporin TIP4.3
Os05g0515900	2.08	Similar to heat- and acid-stable phosphoprotein
Os05g0247100	2.08	Drought-induced protein 3; DIP3
Os02g0753800	2.07	Similar to annexin p35; OsANN2
Os12g0538700	2.07	Glutaredoxin 29; OsGRX29
Os06g0158900	2.04	Similar to multidrug-resistance associated protein 3

Os03g0163300	2.03	Glutathione reductase 1
Os01g0175600	2.02	Boron (B) transporter; OsBOR2
Os08g0112700	2.02	Similar to TAGL12 transcription factor
Os11g0143500	2.00	Similar to L-galactono-1,4-lactone dehydrogenase; GalDH

Table S4. ClueGO classification of the genes upregulated in transgenic (TR1) plants.

GO ID	GO Term	Ontology source	Associated genes
GO:0000302	Response to reactive oxygen species	BP	LOC4329643, LOC4332360, LOC4332361, LOC4332474
GO:0004130	Cytochrome-c peroxidase activity	MF	LOC4329643, LOC4332474
GO:0016688	L-ascorbate peroxidase activity	MF	LOC4329643, LOC4332474
GO:0042542	Response to hydrogen peroxide	BP	LOC4332360, LOC4332361
GO:0042744	Hydrogen peroxide catabolic process	BP	LOC4329643, LOC4332474
GO:0098869	Cellular oxidant detoxification	BP	LOC4329643, LOC4332474
GO:0045471	Response to ethanol	BP	LOC4332360, LOC4332361
GO:0046686	Response to cadmium ion	BP	LOC4332360, LOC4332361
GO:0046688	Response to copper ion	BP	LOC4332360, LOC4332361
GO:0051188	Cofactor biosynthetic process	BP	LOC4326739, LOC4326901, LOC4328930, LOC4337415
GO:0009108	Coenzyme biosynthetic process	BP	LOC4326739, LOC4328930
GO:0031967	Organelle envelope	CC	LOC4326901, LOC4329643, LOC4333985, LOC4340941, LOC4343584, LOC4349749
GO:0015994	Chlorophyll metabolic process	BP	LOC4326901, LOC4337415, LOC4343584
GO:0009528	Plastid inner membrane	CC	LOC4326901, LOC4343584
GO:0009941	Chloroplast envelope	CC	LOC4326901, LOC4329643, LOC4333985, LOC4340941, LOC4343584
GO:0005525	GTP binding	MF	LOC4325879, LOC4337890, LOC4338790
GO:0008299	Isoprenoid biosynthetic process	BP	LOC4338768, LOC4343523
GO:0009966	Regulation of signal transduction	BP	LOC4331833, LOC9267696

GO:0004553	Hydrolase activity, hydrolyzing O-glycosyl compounds	MF	LOC4329920, LOC4343697, LOC4344146, LOC4345995, LOC4349267
GO:1901565	Organonitrogen compound catabolic process	BP	LOC4329920, LOC4343584, LOC4349267
GO:0004568	Chitinase activity	MF	LOC4329920, LOC4349267
GO:0016998	Cell wall macromolecule catabolic process	BP	LOC4329920, LOC4349267
GO:0102483	Scopolin beta-glucosidase activity	MF	LOC4344146, LOC4345995
GO:0008422	Beta-glucosidase activity	MF	LOC4344146, LOC4345995
GO:0006032	Chitin catabolic process	BP	LOC4329920, LOC4349267
GO:0019725	Cellular homeostasis	BP	LOC4324276, LOC4325734, LOC4330049, LOC4330468, LOC4340941, LOC4343119, LOC4343348, LOC4352426
GO:0045454	Cell redox homeostasis	BP	LOC4325734, LOC4330468, LOC4343348, LOC4352426
GO:0006833	Water transport	BP	LOC4324276, LOC4330049, LOC4340941, LOC4343119
GO:0015250	Water channel activity	MF	LOC4324276, LOC4330049, LOC4340941, LOC4343119
GO:0009992	Cellular water homeostasis	BP	LOC4324276, LOC4330049, LOC4340941, LOC4343119
GO:0015254	Glycerol channel activity	MF	LOC4324276, LOC4330049, LOC4340941, LOC4343119
GO:0015793	Glycerol transport	BP	LOC4324276, LOC4330049, LOC4340941, LOC4343119
GO:0009705	Plant-type vacuole membrane	CC	LOC4324276, LOC4340941
GO:0042807	Central vacuole	CC	LOC4324276, LOC4340941
GO:0044283	Small molecule biosynthetic process	BP	LOC4326739, LOC4338768, LOC4343348, LOC4344361, LOC4349749
GO:0042364	Water-soluble vitamin biosynthetic process	BP	LOC4326739, LOC4338768, LOC4349749
GO:0019725	Cellular homeostasis	BP	LOC4324276, LOC4325734, LOC4330049, LOC4330468, LOC4340941, LOC4343119, LOC4343348, LOC4352426
GO:0045454	Cell redox homeostasis	BP	LOC4325734, LOC4330468, LOC4343348, LOC4352426

GO:0015035	Protein disulfide oxidoreductase activity	MF	LOC4325734, LOC4352426
GO:0005385	Zinc ion transmembrane transporter activity	MF	LOC4326901, LOC9271816
GO:0071577	Zinc II ion transmembrane transport	BP	LOC4326901, LOC9271816
GO:0071822	Protein complex subunit organization	BP	LOC4325697, LOC4334594, LOC4343584
GO:0000603	Glycosphingolipid biosynthesis	KEGG	LOC4343697, LOC4344290, LOC4348988

BP, biological process; MF, molecular function; CC, cellular component.

LOC number was identified by Quick GO (<https://www.ebi.ac.uk/QuickGO/>) which is a fast browser for Gene Ontology terms and annotations.

Table S5. Genes downregulated in paddy field-grown transgenic rice (TR1) plants.

Gene symbol	Intensity	Gene information
Os11g0188850	0.01	RNA-directed DNA polymerase
AK062276.1	0.01	F-box domain containing protein
Os11g0182500	0.01	Ankyrin repeat domain containing protein; OsPIANK1
Os06g0185400	0.05	Conserved hypothetical protein
CB656443	0.06	Cysteine protease 1 precursor
Os02g0582600	0.10	Heavy metal transport/detoxification protein domain containing protein
Os02g0187800	0.17	Cinnamyl alcohol dehydrogenase
Os06g0579200	0.19	Low-affinity cation transporter
Os09g0417600	0.23	WRKY transcription factor 76; OsWRKY76
Os01g0816700	0.24	Similar to L-ascorbate oxidase homolog precursor
Os08g0157500	0.25	Quercetin 3-O-methyltransferase 1; COMT
Os09g0532900	0.27	Transcription factor MYB42/85; OsMYB42/85
Os04g0675400	0.27	DnaJ domain protein C45; OsDjC45
Os05g0322900	0.28	Similar to WRKY transcription factor 45; OsWRKY45
Os09g0456800	0.28	Similar to heat stress transcription factor Spl7 (Heat shock transcription factor) (Heat shock factor RHSF10)
Os03g0179400	0.29	Drought-inducible receptor-like cytoplasmic kinase, Drought tolerance, Regulator of grain yield
Os04g0434800	0.29	Similar to thylakoid-bound ascorbate peroxidase; OsAPx7
Os11g0199700	0.30	Similar to VHS and GAT domain protein; similar to seed protein B32E
Os06g0157700	0.30	Florigen, heading date; flowering locus T
Os06g0111600	0.30	Serine/threonine protein kinase-related domain containing protein; OsWAK11
Os10g0552400	0.30	Zinc finger, RING/FYVE/PHD-type domain containing protein; OsPUB67

Os11g0490900	0.32	Similar to WRKY transcription factor 72; OsWRKY72
Os04g0675400	0.32	Similar to chaperone protein dnaJ; OsDjC45
Os01g0134700	0.32	Calmodulin binding protein-like family protein
LOC_Os01g06900	0.32	Verticillium wilt disease resistance protein Ve2
Os03g0187400	0.33	Zinc finger, FYVE/PHD-type domain containing protein
Os06g0232300	0.35	Similar to auxin transport protein REH1
Os01g0289666	0.35	WRKY9
Os09g0532000	0.36	TonB box, conserved site domain containing protein; stay green leaf(t), stay green, staying green during senescence
Os11g0597700	0.36	Similar to resistant protein
Os07g0683200	0.37	Similar to NAC domain transcription factor; ONAC103
Os01g0141000	0.37	APETALA2/ethylene-responsive element binding protein 129; AP2/EREBP#129
Os06g0671600	0.37	Small auxin-up RNA 26; OsSAUR26
Os01g0194600	0.37	Glutaredoxin 2; OsGRX2
Os01g0971800	0.37	Similar to two-component response regulator ARR11
Os01g0178900	0.37	Similar to symbiosis-related disease resistance protein
Os03g0626700	0.38	Multi antimicrobial extrusion protein MatE family protein
Os07g0680400	0.39	DNA-binding WRKY domain containing protein; OsWRKY47
Os06g0143100	0.39	Similar to cadmium tolerant 1
Os09g0313600	0.40	Disease resistance protein domain containing protein
Os02g0303500	0.40	Eggshell protein family protein
Os11g0184900	0.41	Similar to OsNAC5 protein
Os11g0126900	0.41	NAC-domain protein, drought tolerance
Os06g0157500	0.41	Florigen, flowering time, long-day promotion
Os05g0120700	0.42	ATPase, P-type, K/Mg/Cd/Zn/Na/Ca/Na/H-transporter family protein
Os07g0665200	0.43	Cu/Zn-superoxide dismutase; Cu/Zn-SOD
Os01g0665500	0.43	Similar to WRKY transcription factor 16; OsWRKY16
Os05g0439000	0.43	Similar to RING-H2 finger protein ATL1O
Os05g0198200	0.43	Glutaredoxin 16; OsGRX16
Os02g0738200	0.43	Zinc finger, RING/FYVE/PHD-type domain containing protein
LOC_Os03g25150	0.43	Flavonoid 3,5-hydroxylase 2
Os03g0341600	0.44	Pollen Ole e 1 allergen/extensin domain containing protein
Os06g0125800	0.44	Zinc finger, RING/FYVE/PHD-type domain containing protein
Os04g0418500	0.44	Similar to photoperiod responsive protein
Os03g0182800	0.44	Similar to ethylene responsive element binding factor3 (OsERF3); EREBP transcription factor
Os01g0135900	0.44	Similar to cytosolic class I small heat shock protein 3C; OsHsp17.9B
Os03g0787000	0.44	Similar to syntaxin
Os06g0218300	0.44	Zinc finger, RING-type domain containing protein
Os05g0549800	0.44	Similar to DNA-binding protein RAV1; APETALA2/ethylene-responsive element binding protein 96 (AP2/EREBP#096)

Os02g0571100	0.44	Terpenoid synthase domain containing protein
Os05g0530400	0.45	Heat stress transcription factor Spl7 (Heat shock transcription factor) (Heat shock factor RHF10); HSF10
Os07g0107300	0.45	Plant disease resistance response protein family protein
Os06g0614100	0.45	bZIP transcription factor, bZIP-1 domain containing protein; OsbZIP49
Os01g0974400	0.45	Zinc finger, RING/FYVE/PHD-type domain containing protein; OsRFPHC-7
LOC_Os09g04430	0.45	Lactoylglutathione lyase
Os01g0563000	0.45	Peptidyl-prolyl cis-trans isomerase, FKBP-type domain containing protein; OsFKBP73
Os01g0949700	0.45	Glutathione S-transferase, C-terminal domain containing protein; OsGSTU7
Os01g0723100	0.45	Senescence-associated family protein
Os01g0933900	0.45	Similar to glutathione transferase III(B); OsGSTF4
Os01g0542700	0.45	bZIP transcription factor, bZIP-1 domain containing protein; OsbZIP04
Os12g0105600	0.45	Similar to phosphate starvation regulator protein (regulatory protein of P-starvation acclimation response Psr1).
Os03g0411100	0.45	Similar to Nuclear Y/CCAAT-box binding factor A subunit NF-YA; OsHAP2E, NF-YA, CBF-B
Os05g0553400	0.45	Similar to Myb-related transcription factor-like protein (MYB transcription factor); OsMYB55, OsMYB-2
Os08g0386200	0.46	WRKY transcription factor 69; OsWRKY69
Os01g0949700	0.46	Glutathione S-transferase, C-terminal domain containing protein; OsGSTU7
Os02g0578400	0.46	Photosystem II oxygen evolving complex protein PsbQ family protein
Os03g0718800	0.46	Similar to physical impedance induced protein
Os06g0701600	0.46	Monovalent cation transporter, Na ⁺ and K ⁺ transport; OsHKT9, OsHKT2;4
Os09g0524800	0.46	Apoptosis regulator Bcl-2 protein, BAG domain containing protein; OsBAG1
Os03g0835900	0.46	Similar to ferredoxin III, chloroplast precursor (Fd III)
Os03g0259100	0.46	Similar to basic blue protein (cusacyanin) (plantacyanin) (CBP)
Os09g0467200	0.47	Similar to glutathione S-transferase GST 23
Os05g0557700	0.47	Conserved hypothetical protein; leaf tip necrosis1, phosphate overaccumulator 2
Os10g0159800	0.48	Alcohol dehydrogenase superfamily, zinc-containing protein
Os02g0105400	0.48	Similar to L-lactate dehydrogenase A
Os07g0608200	0.49	Similar to CCAAT-binding transcription factor subunit B; CBF-B
Os05g0537100	0.49	WRKY transcription factor 10
Os01g0246700	0.49	Similar to WRKY transcription factor 1; OsWRKY1
Os06g0292400	0.49	Embryogenesis transmembrane protein
Os01g0225000	0.49	Mitochondrial carrier protein domain containing protein

Intensity is represented as a Log₂ ratio.

Table S6. ClueGO classification of genes downregulated in transgenic (TR1) plants.

GO ID	GO Term	Ontology source	Associated genes
GO:0006082	Organic acid metabolic process	BP	LOC4328012, LOC4330034, LOC4330040, LOC4330649, LOC433014, LOC433918, LOC4334487, LOC4337165, LOC4341343, LOC4343946
GO:0016053	Organic acid biosynthetic process	BP	LOC4330034, LOC4330040, LOC4330649, LOC4333014, LOC4333918, LOC4337165, LOC4341343, LOC4343946
GO:0006520	Cellular amino acid metabolic process	BP	LOC4330034, LOC4330040, LOC4330649, LOC4333014, LOC4333918, LOC4337165, LOC4343946
GO:0009073	Aromatic amino acid family biosynthetic process	BP	LOC4333014, LOC4333918, LOC4337165, LOC4343946
GO:0019752	Carboxylic acid metabolic process	BP	LOC4328012, LOC4330034, LOC4330040, LOC4330649, LOC4333014, LOC4333918, LOC4334487, LOC4337165, LOC4343946
GO:1901607	Alpha-amino acid biosynthetic process	BP	LOC4330649, LOC4333918
GO:0006541	Glutamine metabolic process	BP	LOC4330649, LOC4333918
GO:0008652	Cellular amino acid biosynthetic process	BP	LOC4330649, LOC4333014, LOC4333918, LOC4337165, LOC4343946
GO:0043648	Dicarboxylic acid metabolic process	BP	LOC4333014, LOC4334487, LOC4337165, LOC4343946
GO:0009423	Chorismate biosynthetic process	BP	LOC4333014, LOC4337165, LOC4343946
GO:0008171	O-methyltransferase activity	MF	LOC4344702, LOC4345934, LOC4345935
GO:0008757	S-adenosylmethionine-dependent methyltransferase activity	MF	LOC4344702, LOC4345934, LOC4345935
GO:0006556	S-adenosylmethionine biosynthetic process	BP	LOC4325163, LOC4337733
GO:0006811	Ion transport	BP	LOC4324220, LOC4329628, LOC4331635, LOC4333298, LOC4334717, LOC4335115, LOC4335729, LOC4336365, LOC4336726, LOC4341805, LOC4346978

GO:0015075	Ion transmembrane transporter activity	MF	LOC4324220, LOC4329628, LOC4333298, LOC4334717, LOC4335729, LOC4336365, LOC4341805, LOC4346978
GO:0055085	Transmembrane transport	BP	LOC4324220, LOC4326735, LOC4329628, LOC4331635, LOC4333298, LOC4334717, LOC4335115, LOC4335729, LOC4336365, LOC4336442, LOC4338107, LOC4340580, LOC4341805, LOC4346978, LOC4349520
GO:0071705	Nitrogen compound transport	BP	LOC4324220, LOC4329628, LOC4334717, LOC4336365, LOC4349520
GO:0031226	Intrinsic component of plasma membrane	CC	LOC4324220, LOC4324284, LOC4329628, LOC4334717, LOC4335115, LOC4336365, LOC4338107, LOC4349520
GO:0006812	Cation transport	BP	LOC4329628, LOC4333298, LOC4334717, LOC4335729, LOC4336365, LOC4336726, LOC4341805, LOC4346978
GO:0034220	Ion transmembrane transport	BP	LOC4324220, LOC4329628, LOC4333298, LOC4334717, LOC4335729, LOC4336365, LOC4341805, LOC4346978
GO:0015695	Organic cation transport	BP	LOC4329628, LOC4334717, LOC4336365
GO:0008519	Ammonium transmembrane transporter activity	MF	LOC4329628, LOC4334717, LOC4336365
GO:0030001	Metal ion transport	BP	LOC4333298, LOC4335729, LOC4336726, LOC4341805, LOC4346978
GO:0071805	Potassium ion transmembrane transport	BP	LOC4333298, LOC4335729, LOC4341805
GO:0072488	Ammonium transmembrane transport	BP	LOC4329628, LOC4334717, LOC4336365
GO:0046873	Metal ion transmembrane transporter activity	MF	LOC4333298, LOC4335729, LOC4341805, LOC4346978
GO:0098869	Cellular oxidant detoxification	BP	LOC4335896, LOC4336726, LOC4345742, LOC4347158
GO:0009570	Chloroplast stroma	CC	LOC4335896, LOC4336726, LOC4347672
GO:0004016	MAPK signaling pathway	KEGG	LOC4324163, LOC4334072, LOC4339397, LOC4339461, LOC4345742, LOC4346736, LOC4347158
GO:0009698	Phenylpropanoid metabolic process	BP	LOC4328485, LOC4328552, LOC4330034, LOC4330040, LOC4345717

GO:0044550	Secondary metabolite biosynthetic process	BP	LOC4328552, LOC4330034, LOC4330040, LOC4341343
GO:0016207	4-coumarate-CoA ligase activity	MF	LOC4328485, LOC4345717
GO:0045548	Phenylalanine ammonia-lyase activity	MF	LOC4330034, LOC4330040
GO:0006559	L-phenylalanine catabolic process	BP	LOC4330034, LOC4330040
GO:0015035	Protein disulfide oxidoreductase activity	MF	LOC4327316, LOC4338050
GO:0015291	Secondary active transmembrane transporter activity	MF	LOC4324220, LOC4331635, LOC4335115, LOC4349520
GO:0005315	Inorganic phosphate transmembrane transporter activity	MF	LOC4331635, LOC4335115
GO:0006820	Anion transport	BP	LOC4324220, LOC4331635, LOC4335115
GO:0006817	Phosphate ion transport	BP	LOC4331635, LOC4335115
GO:0016567	Protein ubiquitination	BP	LOC4324295, LOC4330663, LOC4339966, LOC4339988
GO:0016602	CCAAT-binding factor complex	CC	LOC4333094, LOC4343866
GO:0031969	Chloroplast membrane	CC	LOC4341343, LOC4347672
GO:0042435	Indole-containing compound biosynthetic process	BP	LOC4333918, LOC4344702
GO:0048573	Photoperiodism, flowering	BP	LOC4340184, LOC4340185
GO:0009909	Regulation of flower development	BP	LOC4340184, LOC4340185

BP, biological process; MF, molecular function; CC, cellular component

LOC number was identified using Quick GO (<https://www.ebi.ac.uk/QuickGO/>), which is a fast browser for Gene Ontology terms and annotations.

Table S7. Functional classification of genes differentially expressed in transgenic rice (TR1) plants.

Functional category	Gene count	Related gene
Apoptotic process	13	Os01g0229400, Os11g0598500, Os03g0254000, Os03g0848700, Os03g0848700, Os10g0124300, Os12g0512400, Os09g0524800, Os11g0579200, Os11g0598300, Os02g0282000, Os11g0226933, Os11g0598500, Os09g0313600
Cell cycle	2	Os12g0143800, Os08g0479300

Cell growth	6	Os07g0679300, Os08g0509200, Os05g0548900, Os07g0148800, Os01g0142800, Os07g0690900
Biological regulation	137	Os01g0106400, Os07g0679300, Os07g0509800, Os01g0229400, Os11g0530600, Os03g0355900, Os01g0124000, Os11g0113700, Os07g0558500, Os03g0640800, Os02g0257300, Os05g0161500, Os11g0222225, Os05g0521300, Os07g0509800, Os04g0683700, Os05g0506000, Os03g0745000, Os03g0764600, Os09g0426800, Os12g0438600, Os05g0575000, Os06g0158900, Os01g0911300, Os05g0217800, etc.
Biosynthetic process	128	Os08g0434100, Os10g0502400, Os03g0856500, Os03g0856500, Os06g0685300, Os07g0509800, Os09g0451400, Os04g0678700, Os11g0530600, Os12g0143800, Os03g0797400, Os03g0640800, Os07g0689600, Os07g0691200, Os07g0691200, Os11g0644800, Os02g0194700, Os02g0257300, Os11g0222225, Os05g0548900, Os01g0812800, Os03g0423300, Os03g0431200, Os11g0491400, Os01g0225400, Os03g0850400, Os09g0294000, etc.
Cell adhesion	8	Os05g0453300, Os07g0148800, Os03g0764600, Os11g0667700, Os01g0911300, Os11g0199700, Os07g0511400, Os09g0364400
Cell differentiation	10	Os07g0679300, Os07g0124100, Os06g0157700, Os06g0157500, Os08g0509200, Os05g0548900, Os07g0148800, Os09g0426800, Os01g0142800, Os02g0122600
Cell development	6	Os07g0679300, Os08g0509200, Os05g0548900, Os07g0148800, Os01g0142800, Os02g0122600
Lipid metabolic process	39	Os06g0685300, Os07g0679300, Os11g0530600, Os03g0355900, Os07g0558500, Os01g0717000, Os03g0700700, Os09g0513100, Os02g0194700, Os02g0257300, Os05g0548900, Os03g0423300, Os02g0589000, Os02g0596000, Os01g0757200, Os07g0546000, Os09g0426800, Os01g0369900, Os02g0571100, Os07g0616200, Os03g0261100, Os05g0363200, Os10g0140300, Os10g0533500, Os07g0598000, Os03g0356582, Os06g0694200, Os06g0569500, Os07g0190000, Os06g0600400, Os05g0408900, Os10g0493600, Os08g0441500, Os07g0412100, Os07g0162100, Os06g0685300
Transport	102	Os08g0434100, Os06g0685300, Os07g0679300, Os01g0229400, Os05g0160300, Os11g0530600, Os07g0558500, Os07g0689600, Os02g0257300, Os03g0638200, Os03g0638200, Os06g0264500, Os02g0596000, Os01g0232000, Os04g0683700, Os06g0566300, Os12g0438600, Os01g0825800, Os04g0612400, Os04g0612400, Os04g0612500, Os04g0612500, Os09g0537700, etc.
Translation	10	Os03g0856500, Os03g0856500, Os01g0812800, Os01g0185200, Os09g0265800, Os09g0294000, Os01g0939500, Os02g0529700, Os03g0816200, Os04g0573200